IN THE SPECIFICATION:

Page 1, line 2, please delete the current title and replace it with the following:

--COMPOSITIONS FOR DETECTING AGGREGATIONS OF PROTEINS--

Page 1, lines 5-8, please replace the paragraph under the heading "Cross-Reference to related Applications" with the following amended paragraph:

--This application is a continuation of U.S. patent application 09/825,244 filed 2 April 2001; which is a continuation-in-part of U.S. application 09/698,846 filed 27 October 2000; which is a continuation-in-part of U.S. application 09/684,386 filed 04 October 2000; which is a continuation-in-part of U.S. application 09/602,586 filed 21 June 2000; which is a continuation-in-part of U.S. application 09/561,579 filed 28 April 2000; which is a continuation-in-part of U.S. application 09/303,029 filed 30 April 1999 ~~09/303,029 filed 30 April 1999; 09/561,579 filed 28 April 2000; 09/602,586 filed 21 June 2000; 09/684,386 filed 04 October 2000; and 09/698,846 filed 27 October 2000~~, all of which are incorporated herein by reference in their entirety.--

Page 4, line 10, immediately below the section heading "Summary of the Invention," please insert the following amended paragraphs from parent application 09/698,846:

--Methods and compounds are provided for multiplexed determinations, where the compounds can be linked to binding compounds for detection of reciprocal binding compounds in a sample. The methods are distinguished by having a plurality of binding events in a single vessel using a mixture of differentially eTag reporter ~~receptor~~ conjugated binding compounds, the release of identifying eTag reporter ~~receptor~~ of those binding compounds bound to their target compounds in the same vessel, and the detection of the released identifying tags by separation of the tags in a single run. The eTag reporter ~~receptor~~ are distinguished by having one or more physical characteristics that allow them to be separated and detected.

The method employs a mixture of binding compounds bound to eTag reporters, where each eTag reporter has a characteristic that allows it to be uniquely detected in a single separation run. The method involves combining the eTag reporter conjugated binding compound with a sample to determine the presence of a plurality of targets under conditions where the binding compounds bind to any reciprocal binding partners to form a binding complex. After sufficient time for binding to occur, the eTag reporters can be released from binding complexes

in the same vessel. Various techniques are employed depending upon the nature of the binding compounds for releasing the eTag reporters bound to the complex. The released eTag reporters are then separated and identified by their differentiable characteristics free of interference from the eTag reporters still bound to the binding compound. The techniques for differentiating between eTag reporters bound to a complex and not bound to a complex, include enzymatic reactions that require the complex to exist for cleavage to occur, modification by using ligand/receptor binding, where the ligand is part of the binding compound, so that after cleavage, eTag reporter ~~receptor~~ still bound to the binding compound is modified, dual binding to the target resulting in release of the eTag reporter ~~receptor~~, where optionally eTag reporter ~~receptor~~ bound to the binding compound is modified, and the like.

One set of eTag reporters ~~receptors~~ are distinguished by differences, which include mass as a characteristic. These eTag reporters do not rely on differentiation based on oligonucleotides of 2 or more, usually 3 or more nucleotides, but rather on organic chemical building blocks that are conveniently combined together to provide for large numbers of differentiable compounds. Therefore, while the original eTag reporter or eTag reporter conjugated to the binding compound can have 2 or more nucleotides, when released from the binding compound, the released eTag reporter will have not more than 3, usually not more than 2 nucleotides. Of particular interest are eTag reporters ~~receptors~~ that are characterized by differences in their mass/charge ratio. These compounds are distinguished by having differences in mobility and are characterized by having regions, which serve as (1) a cleavable linking region; (2) a mass-modifying region; (3) a charge-modifying region: and (4) a detectable region, where the regions may be separate and distinct or combined, there being at least two distinct regions that provide for the differentiation. These eTag reporters may be combined in kits and assays with compounds having all of the regions within a single region to further expand the number of different compounds used as eTag reporters in a multiplexed determination. These compounds find use with other compounds where the different regions are present in the same moiety, for example one to two regions, where the charge-modifying region may also be the detectable region or the mass-modifying region. By having a plurality of compounds that can serve as identifying molecules, mixtures of target compounds can be assayed in a single vessel. By using protocols that result in the release of eTag™ reporters from the binding compound that are identifiable due to differences in mobility, the analysis is greatly simplified, since the eTag reporters will be substantially free of interfering materials and their differences in mobility will allow for accurate detection and quantitation.—

Page 5, lines 39-41, please replace the caption for Figures 1A, B and C with the following:

--Figures 1A, B and C depict the snp detection sequences for two snp alleles (Fig. 1A) ~~(A)~~ (SEQ ID NO: 3, SEQ ID NO: 52, SEQ ID NO: 4, and SEQ ID NO: 53, from top to bottom, respectively), the optical characteristics of the fluorescent dyes (Fig. 1B) ~~(B)~~, and the cleaved fragments from the snp detection sequences (Fig. 1C) ~~(C)~~. --

Page 6, lines 9-10, please replace the caption for Figure 4 with the following:

--Figure 4 illustrates the design and synthesis of e-tags using ~~a LabCard (Detection: 4.7 cm; 200 V/cm) and~~ standard phosphoramidite coupling chemistry.—

Page 8, lines 16-17, please replace the caption for Figure 33 with the following:

--Figure 33 is a schematic diagram of the steps involved in the synthesis of the phosphoroamidite of biotin-deoxycytosine (dC)~~(Reagent C)~~.--

Page 8, lines 16-17, please replace the caption for Figure 33 with the following:

--Figure 34 is a schematic diagram of the steps involved in the synthesis of the phosphoroamidite of biotin-deoxyadenosine (dA)~~ (Reagent D)~~.--

Page 8, line 21, immediately below the section heading "Description of the Specific Embodiments," please insert the following paragraphs from parent application 09/698,846:

--Instead of nucleic acid assays, one may be interested in protein assays. For determining a mixture of proteins, one may use intact cells, intact viruses, viral infected cells, lysates, plastids, mitochondria or other organelles, fractionated samples, or other aggregation of proteins, by themselves or in conjunction with other compounds. Any source of a mixture of proteins can be used, where there is an interest in identifying a plurality of proteins.

Proteomics has come to the fore, where one is interested in cellular expression during metabolism, mitosis, meiosis, in response to an external stimulus, e.g. drug, virus, change in physical or chemical condition, involving excess or deficient nutrients and cofactors, stress,

aging, presence of particular strains of an organism and identifying the organism and strain, multiple drug resistance, and the like. It is necessary to have a means for identifying a large number of proteins in a single sample, as well as providing some quantitation of the different proteins being detected. In one assay one may use binding proteins specific for the target proteins. One group of binding proteins is bound to a support, such as a vessel or channel wall, particles, magnetic or non-magnetic, e.g. latex particles, dextrose, sepharose, cellulose, etc., where the support permits sequestering the target proteins to the support. Most commonly, antibodies, particularly monoclonal antibodies rather than antisera, will be used, although the latter may also find use. In some situations other receptors may find use, such as lectins, enzymes, surface membrane proteins, etc. and in some situations, ligands for the proteins may be employed. The reciprocal-binding members, receptors and ligands, may be bound to the support through covalent or non-covalent bonding. Activated surfaces find use, where the surface has an active functional group that will react with the reciprocal-binding member to provide for stable binding to the surface, e.g. silyl chloride modified glass, cyanogen bromide modified polysaccharides, etc. Proteins bind tightly to some plastic surfaces, so that no covalent bonding is required. Ligands have or can be provided with active functional groups for bonding to the surface. If desired the binding to the surface can be accomplished in two steps by bonding a ligand to the reciprocal binding member and binding a ligand binding member to the support, for example, biotin as the ligand and strept/avidin as the ligand binding member, or one may have anti-Ig bound to the surface to bind to antibodies bound to the target protein. In addition, where a change in environment is localized, one may have a large concentration of a counteracting agent, e.g. a large amount of buffer at pH 7, for example, $\geq$200mM phosphate, where ammonia is produced that creates a localized basic environment.

The sample is combined with the reciprocal binding member, which may be bound to the support or subsequently bound to the support. After washing away the other components of the mixture, receptor for the target protein labeled with eTag reporter molecules specific for the particular receptor are added to the bound target protein, so as to become bound to the support through the target protein. One or more eTag reporter molecules will be bound to the receptor, usually not more than about 20, frequently not more than about 10. The number will be limited by the degree of loss of the binding affinity as the number of eTag reporter molecules is increased. Normally, the support bound receptor and the eTag reporter labeled receptor will bind to different epitopes of the target protein, although in some situations where the target has a

plurality of the same epitope, the receptors may be specific for the same epitope. After washing away all eTag reporter labeled receptor that is not specifically bound to the target protein(s), the eTag reporter molecules are released and assayed.

Where the target permits binding of two reciprocal binding members or where an additional reagent is provided which permits this event, one can use determinations involving "channeling" or energy transfer. See, for example, U.S. Patent nos. 5,843,666 and 5,573,906. There are numerous methodologies involving channeling in the literature, where for the most part, the channeling was involved in producing a directly detectable signal, usually a change in absorption or emission of light. Channeling involves having two reagents, where the first reagent, when in proximity to the second reagent, produces a detectable signal. For the eTag reporter, the detectable signal is the release of the eTag reporter from the binding component. The release will usually be a function of the production of a short-lived entity, such as a chemical species or a photoactivated excited species, but may be the result of changing the local environment as compared to the bulk solution. So far as the chemical species, illustrative species include singlet oxygen, hydrogen peroxide, NADH, and hydroxyl radicals. Two entities are employed that have reciprocal binding members that bind to the same target moiety. One of the entities generates an active species. The other entity has a susceptible functionality that interacts with the active species resulting in release of the eTag reporter or responds to the changed local environment to release the eTag reporter. Either the active species is short lived, so that it will not create significant background because beyond its vicinity, the active species becomes inactive or a scavenger is employed that efficiently scavenges the active species, so that it is not available to react with the susceptible functionality that is not bound to the target.

Generators of reactive species include enzymes, such as oxidases, such as glucose oxidase, xanthene oxidase, D-amino acid oxidase, NADH-FMN oxidoreductase, galactose oxidase, glyceryl phosphate oxidase, sarcosine oxidase, choline oxidase and alcohol oxidase, that produce hydrogen peroxide, horse radish peroxidase, that produces hydroxyl radical, various dehydrogenases that produce NADH or NADPH, urease that produces ammonia to create a high local pH. One cleavable link can be based on the oxidation of sulfur or selenium, where a thioether, sulfoxide, or selenium analog thereof, is present at the α- or β-position in relation to an activating group, which makes the hydrogen α to the activating group acidic and capable of being removed by base, so as to release the oxidized functionality to which is attached the eTag

reporter or to be subject to oxidation with release of the eTag reporter. Alternatively, one may use metal chelates that are stable at one oxidation state and unstable at another oxidation state. Other compounds include α-substituted methylquinones, which have an eTag reporter bonded through a leaving group, such as sulfonyl, oxy, amino, etc.

By using a heterogeneous system, a first agent for causing cleavage may be bound to a surface to provide an environment for release of the eTag reporter when bound to the surface. Where a second agent is required to cause the release of the eTag reporter, the second agent is added after sufficient time for the eTag reporter conjugated binding compound to become bound to the surface. Where the target is a nucleic acid, the nucleic acid may be bound to the first agent containing surface by having ssDNA binding proteins bound to the surface or other convenient means known in the art. Once the target is bound to the surface, the eTag reporter conjugated oligonucleotides homologous the target nucleic acid sequences are added, followed by the second agent. With ligands and proteins, one can have receptors, which bind at one site, on the surface and eTag reporter binding compounds that bind at a different site forming what is referred to in the art as a "sandwich."

For singlet oxygen, one may use various sensitizers, such as squarate derivatives. See, for example, Ullman, et al., Proc. Natl. Acad. Sci. USA 91, 5426-5430 (1994). Examples of combinations that find use in this invention may be found in U.S. Patent nos. 5,536,498; 5,536,834; references cited therein; H.H.Wasserman and R.W.Murray. Singlet Oxygen. Academic Press, New York (1979); A.L.Baumstark, Singlet Oxygen, Vol. 2, CRC Press Inc., Boca Raton, FL 1983. Other cleavage mechanisms may be found in WO99/64519; WO99/13108; WO98/01533 and WO97/28275.

Singlet oxygen reacts with a wide variety of double bonds, with cleavage of the double bond to an oxo group with separation of the eTag reporter. Illustrative olefins include vinyl sulfides, vinyl ethers, enamines, imines substituted at the carbon atoms with an α-methine (CH, a carbon atom having at least one hydrogen atom), where the vinyl group may be in a ring, the heteroatom may be in a ring, or substituted on the cyclic olefinic carbon atom, and there will be at least one and up to four heteroatoms bonded to the olefinic carbon atoms. The resulting dioxetane may decompose spontaneously, by heating above ambient temperature, usually below about 75°C, reaction with acid or base, or photolytically in the absence or presence of a

sensitizer. Numerous articles describe a variety of compounds that can be decomposed with singlet oxygen, where the articles are frequently interested in light emission, so that the compounds have more complicated structures than are required for the subject purposes, where only cleavage is required for release of the eTag reporter from the binding compound. Therefore, for the most part, synthetic convenience, stability under the conditions of the linking to the binding compound and conditions of the binding, and efficiency of release will be the primary factors in selecting a particular structure.

Articles of interest which are illustrative of a much larger literature include: Adam and Liu, J. Amer. Chem. Soc. 94, 1206-1209, 1972, Ando, et al., J.C.S. Chem. Comm. 1972, 477-8, Ando, et al., Tetrahedron 29, 1507-13, 1973, Ando, et al., J. Amer. Chem. Soc. 96, 6766-8, 1974, Ando and Migita, ibid 97, 5028-9, 1975, Wasserman and Terao, Tetra. Lett. 21, 1735-38, 1975, Ando and Watanabe, ibid 47, 4127-30, 1975, Zaklika, et al., Photochemistsry and Photobiology 30, 35-44, 1979, and Adam, et al., Tetra. Lett. 36, 7853-4, 1995. See also, U.S. Patent no. 5,756,726.

The formation of dioxetanes is obtained by the reaction of singlet oxygen with an activated olefin substituted with an eTag reporter at one carbon atom and the binding compound at the other carbon atom of the olefin. See, for example, U.S. Patent no. 5,807,675. These compounds may be depicted by the following formula:

$$(\text{eTag reporter} - W)(X)_nC_\alpha = C_\beta(Y)(Z)$$

wherein:

W may be a bond, a heteroatom, e.g. O, S, N, P, M (intending a metal that forms a stable covalent bond), or a functionality, such as carbonyl, imino, etc., and may be bonded to X or C_α;

at least one X will be aliphatic, aromatic, alicyclic or heterocyclic and bonded to C_α through a hetero atom, e.g. N, O, or S and the other X may be the same or different and may in addition be hydrogen, aliphatic, aromatic, alicyclic or heterocyclic, usually being aromatic or aromatic heterocyclic wherein one X may be taken together with Y to form a ring, usually a heterocyclic ring, with the carbon atoms to which they are attached, generally when other than

hydrogen being from about 1 to 20, usually 1 to 12, more usually 1 to 8 carbon atoms and one X will have 0 to 6, usually 0 to 4 heteroatoms, while the other X will have at least one heteroatom and up to 6 heteroatoms, usually 1 to 4 heteroatoms;

Y will come within the definition of X, usually being bonded to C_β through a heteroatom and as indicated may be taken together with X to form a heterocyclic ring;

Z will usually be aromatic, including heterocyclic aromatic, of from about 4 to 12, usually 4 to 10 carbon atoms and 0 to 4 heteroatoms, as described above, being bonded directly to C_β or through a heteroatom, as described above;

n is 1 or 2, depending upon whether the eTag reporter is bonded to C_α or X;

wherein one of Y and Z will have a functionality for binding to the binding member or be bound to the binding member.

While not depicted in the formula, one may have a plurality of eTag reporters in a single molecule, by having one or more eTag reporters joined to one or both Xs.

Illustrative compounds include S-(eTag reporter) 3-thiolacrylic acid, N-(eTag reporter), N-methyl 4-amino-4-butenoic acid, O-(eTag reporter), 3-hydroxyacrolein, N-(4-carboxyphenyl) 2-(eTag reporter) imidazole, oxazole, and thiazole.

Also of interest are N-alkyl acridinyl derivatives, substituted at the 9 position with a divalent group of the formula: -(CO) X^1 (A) –

wherein:

X^1 is a heteroatom selected from the group consisting of O, S, N, and Se, usually one of the first three; and

A is a chain of at least 2 carbon atoms and usually not more than 6 carbon atoms substituted with an eTag reporter, where preferably the other valences of A are satisfied by hydrogen, although the chain may be substituted with other groups, such as alkyl, aryl, heterocyclic, etc. groups, A generally being not more than 10 carbon atoms.

Also of interest are heterocyclic compounds, such as diheterocyclopentadienes, as exemplified by substituted imidazoles, thiazoles, oxazoles, etc., where the rings will usually be

substituted with at least one aromatic group and in some instances hydrolysis will be necessary to release the eTag reporter.

Also of interest are tellurium (Te) derivatives, where the Te is bonded to an ethylene group having a hydrogen atom β to the Te atom, wherein the ethylene group is part of an alicyclic or heterocyclic ring, that may have an oxo group, preferably fused to an aromatic ring and the other valence of the Te is bonded to the eTag reporter. The rings may be coumarin, benzoxazine, tetralin, etc.—

Page 27, lines 13-19, please replace the indicated paragraph with the following:

-- In one approach, the e-tag probe is constructed sequentially from a single or several monomeric phosphoramidite building blocks (one containing a dye residue), which are chosen to generate tags with unique electrophoretic mobilities based on their mass to charge ratio. The e-tag probe is thus composed of monomeric units of variable charge to mass ratios bridged by phosphate linkers. Figure 4 illustrates the design and synthesis of e-tags using ~~a LabCard (Detection: 4.7 cm; 200 V/cm) and~~ standard phosphoramidite coupling chemistry.[[~~).~~]] The separation of e-tags on a LabCard (Figure 5) has been demonstrated.--

Page 42, lines 20-43, please replace the indicated paragraph with the following:

-- In one SNP determination protocol, the primer includes the complementary base of the SNP. This protocol is referred to as Invader™ technology, and is described in U.S. Patent no. 6,001,567. The protocol involves providing: (a) (i) a cleavage means, which is normally an enzyme, referred to as a Cleavase enzyme ~~cleavase~~, that recognizes a triplex consisting of the target sequence, a primer which binds to the target sequence and terminates at the SNP position and a labeled probe that binds immediately adjacent to the primer and is displaced from the target at the SNP position, when a SNP is present. The Cleavase enzyme ~~cleavase~~ clips the labeled probe at the site of displacement, releasing the label, (ii) a source of target nucleic acid, the target nucleic acid having a first region, a second region and a third region, wherein the first region is downstream from the second region and the second region is contiguous to and downstream from the third region, and (iii) first and second oligonucleotides having 3' and 5' portions, wherein the 3' portion of the first oligonucleotide contains a sequence complementary to the third region of

the target nucleic acid and the 5' portion of the first oligonucleotide and the 3' portion of the second oligonucleotide each contain sequences usually fully complementary to the second region of the target nucleic acid, and the 5' portion of the second oligonucleotide contains sequence complementary to the first region of said target nucleic acid; (b) mixing, in any order, the cleavage means, the target nucleic acid, and the first and second oligonucleotides under hybridization conditions that at least the 3' portion of the first oligonucleotide is annealed to the target nucleic acid and at least the 5' portion of the second oligonucleotide is annealed to any target nucleic acid to from a cleavage structure, where the combined melting temperature of the complementary regions within the 5' and 3' portions of the first oligonucleotide when annealed to the target nucleic acid is greater than the melting temperature of the 3' portion of the first oligonucleotide and cleavage of the cleavage structure occurs to generate labeled products; and (c) detecting the labeled cleavage products. --

Page 61, lines 27-40, please replace the indicated paragraph with the following:

-- In this study, reactions involved a plurality of probes in the same PCR reaction mixture for different SNPs in the gene for the Cystic Fibrosis transmembrane conductance regulator (CFTR). *Taq* DNA Polymerase exhibits 5' to 3' exonuclease activity, causing degradation of an e-tag probe hybridized to template DNA at the 3' end of a PCR primer. In the subject example, sequence-specific e-tag probes with a fluorescent dye attached to the 5' terminus of the probe were employed. PCR was performed with these probes, followed by separation by gel-based capillary electrophoresis to determine cleavage of the e-tag probe. Table 5 indicates the mutation name, exon location, and the nucleotide change and position of the snp in the CFTR sequence. The name of the oligonucleotide reagents, including e-tag probes and PCR primers, are indicated for each snp locus. Two PCR primers were generated to amplify each snp locus, where F indicates the primer in the forward direction, and R indicates the primer in the reverse direction. Two e-tag probes were generated for each snp locus - one hybridizing in the sense direction and one in the antisense direction, indicated as "s" or "as," respectively. The sequence ID numbers of each of these primers and probes are given in Table 5 ~~Table 6~~.

Page 61, line 43, to page 62, line 5, please delete Table 5 and Table 6 and insert in their place the following new Table 5:

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-- Table 5. CFTR snps, e-tag Probes, and PCR Primers

Name	Location_SNP	Mutation	SNP	SEQ ID NOs
CF1	Exon 11	R553X	C1789T	
CF2	Exon 19	R1162X	C3616T	
CF4	Exon 3	G85E	G386A	
CF5	Exon 4	R117H	G482A	
CF6	Exon 7	R347P	G1172C	
CF7	Exon 10	V520F	G1690T	
CF8	Exon 11	G542X	G1756T	
CF9	Exon 11	G551D	G1784A	
CF10*	Exon 11	R560T	G1811C	
CF11*	Exon 18	D1152H	G3586C	
CF13*	Exon 22	G1349D	G4178A	
Name	Hyb_probe_length	Probe_seq	Probe_antisense	
CF1HYB	26	GTGGAGGTCAACGAGCAAGAATTTCT	AGAAATTCTTGCTCGTTGACCTCCAC	SEQ ID NO: 8 and SEQ ID NO: 9
CF2HYB	25	AGATGCGATCTGTGAGCCGAGTCTT	AAGACTCGGCTCACAGATCGCATCT	SEQ ID NO: 10 and SEQ ID NO: 11
CF4HYB	32	TTCTGGAGATTTATGTTCTATGGAATCTTTTT	AAAAAGATTCCATAGAACATAAATCTCCAGAA	SEQ ID NO: 12 and SEQ ID NO: 13
CF5HYB	21	AAGGAGGAACGCTCTATCGCG	CGCGATAGAGCGTTCCTCCTT	SEQ ID NO: 14 and SEQ ID NO: 15
CF6HYB	20	ATTGTTCTGCGCATGGCGGT	ACCGCCATGCGCAGAACAAT	SEQ ID NO: 16 and SEQ ID NO: 17
CF7HYB	25	ATACAGAAGCGTCATCAAAGCATGC	GCATGCTTTGATGACGCTTCTGTAT	SEQ ID NO: 18 and SEQ ID NO: 19
CF8HYB	29	CAATATAGTTCTTGGAGAAGGTGGAATCA	TGATTCCACCTTCTCCAAGAACTATATTG	SEQ ID NO: 20 and SEQ ID NO: 21
CF9HYB	26	CTGAGTGGAGGTCAACGAGCAAGAAT	ATTCTTGCTCGTTGACCTCCACTCAG	SEQ ID NO: 22 and SEQ ID NO: 23
CF10HYB*	32	TTCCATTTTCTTTTTAGAGCAGTATACAAAGA	TCTTTGTATACTGCTCTAAAAAGAAAATGGAA	SEQ ID NO: 24 and SEQ ID NO: 25
CF11HYB*	28	AAACTCCAGCATAGATGTGGATAGCTTG	CAAGCTATCCACATCTATGCTGGAGTTT	SEQ ID NO: 26 and SEQ ID NO: 27
CF13HYB*	23	CTAAGCCATGGCCACAAGCAGTT	AACTGCTTGTGGCCATGGCTTAG	SEQ ID NO: 28 and SEQ ID NO: 29
Name	product_size	forward_seq	Reverse_seq	
CF1PF/R	198	CCTTTCAAATTCAGATTGAGCATAC	TTTACAGCAAATGCTTGCTAGAC	SEQ ID NO: 30 and SEQ ID NO: 31
CF2PF/R	127	TGTGAAATTGTCTGCCATTCTTA	GGTTTGGTTGACTTGGTAGGTTTA	SEQ ID NO: 32 and SEQ ID NO: 33
CF4PF/R	239	TCTTTTGCAGAGAATGGGATAGA	TGGAGTTGGATTCATCCTTTATATT	SEQ ID NO: 34 and SEQ ID NO: 35
CF5PF/R	151	CCAAAGCAGTACAGCCTCTCTTA	CCAAAAATGGCTGGGTGTAG	SEQ ID NO: 36 and SEQ ID NO: 37

CF6PF/R	137	TCTGTGCTTCCCTATGCACTAA	CCAAGAGAGTCATACCATGTTTGTA	SEQ ID NO: 38 and SEQ ID NO: 39
CF7PF/R	146	TGGAGCCTTCAGAGGGTAAA	TGCTTTGATGACGCTTCTGTA	SEQ ID NO: 40 and SEQ ID NO: 41
CF8PF/R	198	CCTTTCAAATTCAGATTGAGCATAC	TTTACAGCAAATGCTTGCTAGAC	SEQ ID NO: 42 and SEQ ID NO: 43
CF9PF/R	198	CCTTTCAAATTCAGATTGAGCATAC	TTTACAGCAAATGCTTGCTAGAC	SEQ ID NO: 44 and SEQ ID NO: 45
CF10PF/R*	108	GACCAGGAAATAGAGAGGAAATGTA	CATCTAGGTATCCAAAAGGAGAGTCTA	SEQ ID NO: 46 and SEQ ID NO: 47
CF11PF/R*	188	GAAGGAGAAGGAAGAGTTGGTATTATC	CGGTATATAGTTCTTCCTCATGCTATT	SEQ ID NO: 48 and SEQ ID NO: 49
CF13PF/R*	138	TTGGGCTCAGATCTGTGATAG	GCAAGATCTTCGCCTTACTG	SEQ ID NO: 50 and SEQ ID NO: 51

Name	Name	Tm_prob,	Tm_forward,	Tm_reverse, oC	forward_length,	reverse_length	
CF1HYB	CF1PF/R	66.83,	60.36,	58.78	25,	23	
CF2HYB	CF2PF/R	68.65,	59.64,	60.51	23,	24	
CF4HYB	CF4PF/R	64.24,	60.21,	59.2	23,	25	
CF5HYB	CF5PF/R	65.06,	60.08,	60.36	23,	20	
CF6HYB	CF6PF/R	68.18,	59.9,	59.48	22,	25	

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